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METHODS

Improving the Results of Statistical Comparison of the Means by Allocation of Animals to Experimental Groups

S. G. Kornilov

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Prior to any investigation, planning has to be carried out. We will call the planning primary if it concerns a study still to be begun, and secondary if we are dealing with a continuation of a study which is currently being performed. The methods of planning are different for different cases.

The present publication is devoted to primary planning.

Since, in contrast to the situation with secondary planning, information on the phenomena of interest is generally not available for primary planning, no recommendations can be offered with respect to the total number of objects (animals, for example) to be involved in the study. This number (N) is to be chosen by the researcher himself or herself, based on intuition and the resources available. The goal of primary planning is to allocate this collection of objects to groups, taking into account that statistical comparison of the means will be the main method of mathematical processing. Primary planning ensures that actually existing differences of the mean will be found with the highest probability.

In the simplest investigation only two groups of objects are involved: control and experimental. But more often than not, there will be a greater number of groups. Let K denote the number of groups. Assume that just one of the characteristics of the objects under study (for example, the weight of the liver) will be analyzed. Let H denote the total number of comparisons to be performed with respect to this parameter (second-order and optional comparisons are not to be included in this number). Let us designate the groups of objects by

Saratov Medical Institute. (Presented by M. Ya. Studenikin, Member of the Russian Academy of Medical Sciences)

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1, 2, 3, ..., K. Take the group with the number i. Then, the number of objects in this group will be

$$n_i = \frac{N}{K+\sqrt{H-h_i}}.$$

Example 1. An experiment is planned in which approximately N=40 animals divided into K=4 groups are involved. Group 1 serves as the control, and the rest of the groups are experimental. A toxic compound is administered to the animals of the experimental groups. During the subsequent stages, all the animals are to be sacrificed, and the liver is to be isolated and weighed for each animal. The experimental scheme presented in Fig. 1, *a* shows that, in addition to comparisons between the control and each of the experimental groups, a comparison between the means in groups 2 and 3 is envisaged. There is a total of H=4 comparisons.

Let us begin the planning with the 1st group. The number of comparisons in which it is involved (in the scheme, the number of lines linking it with the rest of the groups) is h_1=3. Inserting all the known variables in the formula will yield the optimal number of animals in group 1: n_1=40/3=13.33=13. Now we will turn to group 2. According to the scheme, the number h_2 of comparisons in which it is involved is 2. According to the formula, the optimal number of animals in group 2 n=40/4=10. The reader will figure out that in the rest of the groups n_3=10 and n_4=8. Hence, the refined total number of animals will constitute 13+10+10+8=41.

We planned an investigation involving comparisons with respect to just one parameter.If there are several characteristics, the most important or typical parameter is to be chosen for planning. But if all the characteristics are important and the schemes of comparison are not uniform for all of them, the planning is to be performed with respect to each separate parameter and the results (not rounded-off) are to be averaged.

Example 2. An experiment is planned in which comparison of the means with respect to two parameters (the weight of the liver and the weight of the kidneys) will be performed. Both characteristics are of equal importance to the researcher. The tentative number of animals N=40.

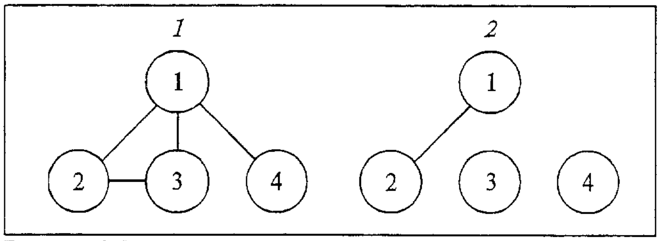

Fig. 1. Scheme of experiments planned in Examples 1 (*a*) and 2 (*b*). Groups of animals are shown as circles. Comparisons of the means are shown by straight lines.

The schemes of comparisons for the weight of the liver and for the weight of the kidneys are presented in Fig. 1, *a* and *b*, respectively. It is seen that the schemes are different: only one comparison between the weight of the kidneys in groups 1 and 2 is required. Although the weight of the kidneys is measured in groups 3 and 4, they are not involved in the comparison (or else the comparisons in which these groups are involved are secondary and/or optional). In the above case, planning is to be performed with respect to each separate parameter, and the results are to be averaged.

The planning for the weight of the liver, performed above, yielded n_1=13.33, n_2=10, n_3=10, and n_4=8. Let us turn to the planning for the weight of the kidneys. Judging by Fig. 1, *b*, the total number of comparisons will be H=1. We will start with the 1st group. The number of comparisons in which it is involved h_1=1. Inserting all the values in the formula yields n_1=40/4=10. The same is true for group 2: n_2=10. Judging by the scheme, the number of comparisons in which the 3rd group is involved is h_3=0. Therefore, the formula yields n_3=40/5=8. Similarly, for group 4 n_4=8.

Hence, we now have two quantities n_i: vis-a-vis the liver n_1= 13.33 and vis-a-vis the kidneys n_1=10. We calculate their arithmetic mean value n_1=(13.33+10)/2=11.67=12. This is to be taken as the number of animals in group 1. Similarly, in the 2nd group n_2=(10+10)/2=10. The results for groups 3 and 4 are n_3=9 and n_4=8. The refined total number of animals will be 12+10+9+8=39. The planning is finished.

The final refined total number of objects may prove to be unsuitable for the researcher. In that case, all the calculations may be repeated using another initial N.